The influence of some starter cultures and GDL on the formation of biogenic amines in dry sausages

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The influence of five common starter cultures and glucono-delta-lactone (GDL) on the formation of histamine, tyramine, putrescine, cadaverine, spermine and spermidine in dry sausages was studied. Sausages were manufactured in a pilot plant from two different batches of raw material. No major differences were observed between the starter cultures studied in the biogenic amine levels detected during ripening. The lowest levels of histamine were detected in sausages fermented by GDL and *Staphylococcae* with or without lactic acid bacteria as a starter culture. In pure culture studies performed with a turbidometric method in MRS broth, non-starter lactic acid bacteria isolated from sausages were found to be more sensitive to acidic conditions than the starter strains used in the study. The addition of 2% histidine to MRS broth resulted in a tremendous increase in histamine production (from 1-2 to 6000 ppm). However, in histidine-fortified MRS broth with GDL addition, only 54 ppm of histamine was formed. According to these results, the pH decrease caused by GDL addition decreases histamine formation in dry sausages and in MRS broth. The differences in pH decrease may be one reason for the very varying concentrations of histamine detected in retail dry sausages.

Keywords: fermentation, histamine, tyramine

Introduction

Over 30 years ago the beneficial influence of added starter cultures was shown to improve and to stabilize the quality of dry sausages. NIINIVAARA (1955) recommended the use of micrococci as a starter culture (resulting in the development of Baktoferment 61) and NURMI (1966) used a combination of *Lactobacillus plantarum* with micrococcae (resulting in the development of Duploferment 66). Since then, many new starter cultures have been developed. However, lactic acid bacteria are still used in most combinations of dry sausage manufacture.

There are several reports of high levels of biogenic amines in fermented sausages obtained from retail markets (RICE et al. 1975, VANDEKERCKHOVE 1977, RAMANTANIS 1982, WORTBERG and WOLLER 1982, PECHANEK et al. 1983, PFANNHAUSER and PECHANEK 1984, BAUER et al. 1989, TSCHABRUN et al. 1990). Factors such as starter cultures, raw material and pH decrease, which affect the formation of biogenic amines during sausage fermentation, are not yet fully understood. It is therefore difficult to make improvements in sausage fermentation as long as the critical points still remain largely unascertained.

Biogenic amines are basic nitrogenous compounds mainly formed by decarboxylation of

amino acids or by amination and transamination of aldehydes and ketones (MAGA 1978, PFANNHAUSER and PECHANEK 1984, ASKAR and TREPTOW 1986). Some aromatic and heterocyclic amines, such as tyramine, histamine and phenylethylamine, have vasoactive properties making their presence in food a potential public health concern (IENISTEA 1971, LÜTHY and SCHLATTER 1983, TAYLOR 1983, ASKAR and TREPTOW 1986, MONERET-VAUTRIN 1985). The aliphatic diamines, putrescine and cadaverine, as well as the polyamines, spermine and spermidine, occur universally in animals and plants, and at least putrescine and spermidine are found in most bacteria. They are important in the regulation of nucleic acid function and protein synthesis, and probably also in the stabilization of membranes (SMITH 1981). They also potentiate the effect of histamine (BJELDANES et al. 1978).

In an earlier study the pH decrease caused by addition of GDL decreased significantly the formation of histamine in minced meat. The lower was the pH on the first day of incubation, the less amines were formed (MAIJALA et al. 1993). GDL hydrolyzes spontaneously in water to gluconic acid and causes a decrease in pH. It is used in the meat industry for dry sausage manufacture, especially with starter cultures which do not contain lactic acid bacteria (LAB). However, the addition of GDL can sometimes be detrimental to flavour and accelerate the development of rancidity (LÜCKE and HECHELMAN 1986). The purpose of this work was to study biogenic amine formation in sausages fermented by five common starter cultures in Finland. The effect of accelerated pH decrease in dry sausages was also studied in the second trial by adding different GDL and glucose combinations to the sausages.

Material and methods

Sausage manufacture

The sausages were prepared from frozen meat originating from one cutter of a commercial meat plant (82 kg pork, 55 pork fat, 69 kg beef and 3% NaCl). The mass was divided into five samples and processed in a Seydelmann cutter (K41) with addition of sodium nitrite ($NaNO_2$) as a 10% aqueous solution (60 ppm), KNO_3 120 ppm, ascorbic acid (0.025%) and spices.

To the sausages fermented by Baktoferment 61 (Rudolf Müller & co., Germany), containing *Staphylococcus carnosus* MIII, 0.7% GDL and 0.15% glucose were added. In the other sausages, 0.6% of glucose (series a) was used together with one of four different starter cultures: Duploferment 66 (Rudolf Müller & co.) containing *S. carnosus* and *Lactobacillus plantarum* (1), Pentoferment 85 (Rudolf Müller & co.) containing *S. carnosus* and *Lactobacillus pentosus* (2), Condi-Rasant 820/10 (Gewürzmüller, Germany) containing *Staphylococcus* spp. and *Pediococcus pentosaceus* (3) or Flora-Carn SL (Chr. Hansen's Lab., Denmark) containing *S. carnosus* and *L. pentosus* (4). The pure LAB strains isolated from these starter cultures had previously been found to be histamine- and tyramine-negative in fortified MRS broth (MAIJALA 1993). The pure strains of *Staphylococcus* were also found to be histamine- and tyramine-negative with a similar method in trypticase soy broth.

This trial was repeated twice. In the second trial, different levels of addition of GDL (Finnsugar Bioproducts, Finland) together with glucose were studied in conjunction with the starter cultures 1-4. Each sample was divided into three subsamples designated a, b and c. No GDL but 0.6% glucose was added to the samples (a) as in the first trial, 0.25% GDL and 0.4% glucose were added to samples (b), and 0.5% GDL and 0.20% glucose to samples (c). The sausages (weight 300 g, diameter 60 mm) were stuffed into fibrous casings and ripened for 2 days at 23°C (93 %rh), 1 day at 21°C (85 %rh) and then for 4 days at 20°C (85 %rh). The remainder of the ripening and storage for up to 7 weeks was at 10°C (70 %rh). The sausages were lightly smoked during days 1-5.

Microbiological and chemical analyses

One sausage from each sausage type (B,1-4) was taken as a sample 6 times during ripening. The

cover was cleaned with 70% ethanol and removed aseptically. The whole sausage was cut into small pieces and homogenized mechanically. Thereafter the mass was mixed well and samples were taken for analysis of biogenic amines and microbiological content and for measurement of pH and a_w.

pH values were measured directly from the samples using an Orion Research Incorporated SA 520 pH/mV meter equipped with a Ross^{TM} pH electrode no. 8163 (Switzerland). After a 2-h adaptation period a_w values were obtained at 25°C from a sample of 25-30 g using a Rotronic Hygroskop (Fattore Vitale & Co, Italy). A 10 g sample of mixed sausage was serially diluted with a diluent containing 0.1% peptone and 0.85% NaCl in sterile deionized water. Coliforms were counted on Violet Red Bile Agar (VRB, Orion; ISO 4832), fecal streptococci on Slanetz-Bartley agar (SB, Oxoid, NCFA 68), moulds and yeasts on Malt extract agar (Oxoid) with added chlortetracycline (100 mg/l) and chloramphenicol (100 mg/l), *Staphylococcae* on blood agar base (BBL) containing 5% defibrinated blood and LAB on de Man, Rogosa and Sharpe agar with sorbic acid (MRS-S, MRS of LAB M with added sorbic acid of Fluka, incubated at 20-22°C for 5 days anaerobically). Biogenic amines were determined using the HPLC method of EEROLA et al. (1993). The detection limits were 1 mg/kg for tyramine, histamine, spermine, spermidine and cadaverine and 2 mg/kg for putrescine.

Histamine production by pure cultures

The influence of low pH achieved with GDL or by the initial addition of lactic acid was studied by a turbidometric method. At the same time the pH values were monitored in similar cultures maintaned in small tubes at 30°C. Histamine was analyzed from tubes by HPLC after 5 days of incubation. Because no major differences were observed between the growth patterns of the four starter cultures, one strain (GS-11) isolated from Duploferment 66 (*L. plantarum*) was selected to represent the starter cultures. Two non-starter lactic acid bacteria (NSLAB) isolated from the sausages during ripening were also used. G-106 produced 930 ppm histamine and 920 ppm tyramine and G-261 produced < 1 ppm histamine and 400 ppm tyramine in fortified MRS broth (MAIJALA 1993).

LAB strains (preserved at -75°C) were incubated in MRS broth (Oxoid) at 30°C for 24 h. 100 µl of this culture was inoculated into a fresh MRS broth and incubated at 30°C for exactly 24 h. These cultures were used for turbidometric inoculations. The cell densities (cfu/ml) of the cultures were determined by culturing serial dilutions on MRS-S plates. 10 µl of a 10^{-1} dilution of MRS-broth cultures ($\log_{10}$ 8 cfu/ml) was added to the wells of Honeycomb plates, each containing 390 µl of basic broth. As a basic broth either pure MRS broth, MRS broth with lactic acid (pH 4.7) or MRS broth with GDL (1% or 2%), all with or without L-histidinemonohydrochloride (Merck 4350), were used. Three replicate wells were used both for the samples and for the negative controls. The cuvettes were incubated in a data logging turbidometer incubator (Bioscreen^{R}, Labsystems Oy, Finland) at 30°C for 120 h. The filter giving the lowest possible background absorbance values was chosen (600 nm) and the optical density was measured at 30 min intervals.

Results

The levels of biogenic amines increased during ripening, as was reported e.g. by RAMANTANIS (1982) (Table 1). In both trials the final levels of tyramine were lowest in the sausages fermented by Baktoferment 61 + GDL. However, the addition of GDL also decreased to some extent the tyramine levels detected in sausages fermented by Duploferment 66 and Flora-Carn SL (Fig. 1).

The influence of the raw material was especially clear on the formation of histamine. In the first trial hardly any histamine was detected but in the second trial the amounts increased up to 108 ppm. The lowest levels of histamine in the second trial were detected in the sausages fermented by Baktoferment 61 + GDL and Flora-Carn SL (Table 1). Histamine levels also clearly decreased when GDL was added (Fig. 2). There were no major differ-

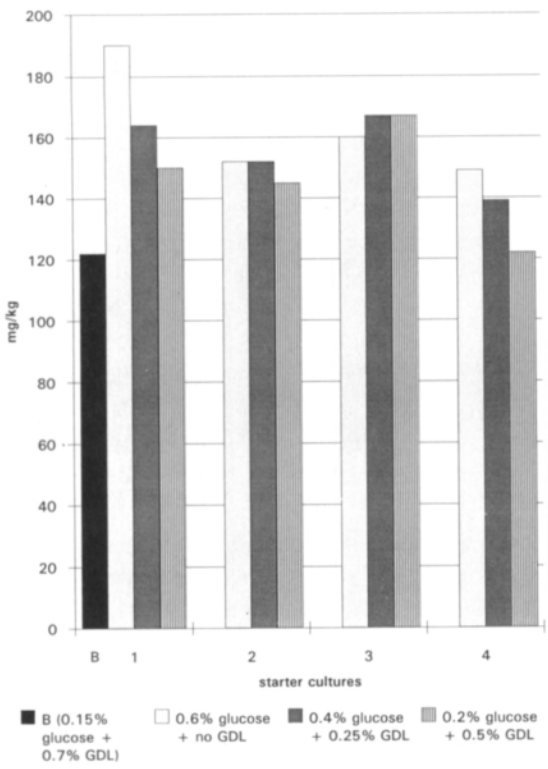

Fig. 1. Tyramine levels detected in sausages fermented with different GDL and glucose combinations. For starter cultures, see Table 2. The results of sausages fermented by B, 1 and 4 have been presented previously (Maijala and Eerola 1993).

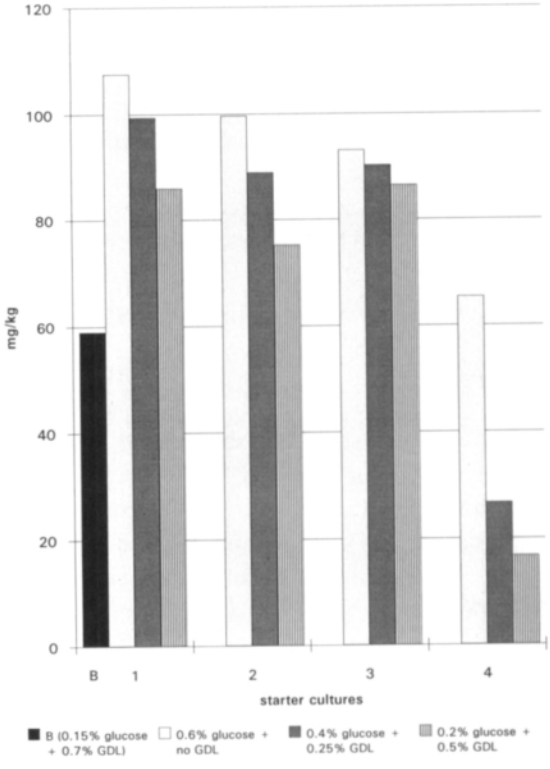

Fig. 2. Histamine levels detected in sausages fermented with different GDL and glucose combinations. For starter cultures, see Table 2. The results of sausages fermented by B, 1 and 4 have been presented previously (Maijala and Eerola 1993).

ences between the starter cultures 1-4 regarding the levles of other amines during ripening.

In both trials the most rapid pH decrease was in the sausages fermented by Baktoferment 61 + GDL, as expected. The addition of GDL with a smaller amount of glucose also lowered the pH values in the beginning of the fermentation (Table 2). There were no great differences between the a_w values of different sausages during ripening. The a_w values decreased from 0.98 to 0.90-0.93 (21 days) and to 0.78-0.84 (49 days) in these trials.

The results of LAB and *Staphylococcus* in sausages fermented with starter cultures 1-4 without GDL addition are presented in Table 3. The levels of coliforms were < log 1.0 except in the raw material of the second trial (log 1.1). The levels of fecal streptococci (< log 3.1), yeasts (< log. 4.7) and *Bacillus* spp. (< log 3.6) also remained at the normal level for this type of product. No moulds were detected in any of the samples. The GDL addition had no influence on the bacterial counts measured.

In pure culture studies the NSLAB strains (G-106 and G-261) decreased the pH slowly as compared with the starter strain GS-11. However, the final pH values with all these strains were almost equal after 5 days of incubation in pure MRS broth (Table 4). GS-11 also grew much better than the NSLAB strains in pure MRS broth (Fig. 3-5). Acidification of MRS broth below pH 5.0 with lactic acid or 1% GDL increased the lag time and decreased the growth rate of the NSLAB strains but not of GS-11. Furthermore, the addition of 2% GDL with or without histidine to MRS broth prevented the growth of NSLAB during five days of

Table 1. Amounts of biogenic amines during the ripening of dry sausages in two trials (mg/kg). B and the numbers 1-4 refer to the different starter cultures used, see Table 2. (a = 0.6% glucose + no glucono-delta-lactone, n.d. = not detected).

		First trial:					Second trial:				
	Day	B	1a	2a	3a	4a	B	1a	2a	3a	4a
Histamine	0	1	1	1	1	1	2	2	2	2	2
	3	2	2	2	3	2	2	5	2	5	4
	7	2	2	2	2	2	1	46	39	41	9
	21	4	4	3	3	3	43	71	73	66	16
	35	5	4	4	4	3	59	104	104	98	29
	49	8	4	4	4	4	59	108	100	93	65
Tyramine	0	n.d.	n.d.	n.d.	n.d.	n.d.	1	1	1	1	1
	3	n.d.	3	5	7	8	12	14	10	13	10
	7	16	59	74	68	71	60	91	102	99	42
	21	37	101	119	107	104	93	117	120	117	86
	35	55	129	143	146	120	113	149	146	144	133
	49	76	146	148	166	129	122	190	152	160	149
Putrescine	0	8	8	8	8	8	17	17	17	17	17
	3	6	5	5	4	4	n.d.	4	4	6	7
	7	5	7	10	13	12	4	2	n.d.	n.d.	2
	21	10	13	15	27	13	5	13	13	5	8
	35	16	16	19	31	13	4	6	5	7	n.d.
	49	31	23	25	41	18	5	9	15	17	17
Cadaverine	0	n.d.	n.d.	n.d.	n.d.	n.d.	1	1	1	1	1
	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2	1	1	1
	7	n.d.	n.d.	n.d.	n.d.	n.d.	2	4	5	5	2
	21	n.d.	2	n.d.	n.d.	1	3	5	6	5	3
	35	n.d.	n.d.	n.d.	n.d.	n.d.	3	8	8	6	4
	49	n.d.	n.d.	n.d.	n.d.	n.d.	4	7	7	5	5
Spermidine	0	3	3	3	3	3	2	2	2	2	2
	3	3	3	3	4	3	3	4	3	3	3
	7	2	2	3	3	4	2	2	2	3	3
	21	3	3	4	4	4	2	3	2	2	3
	35	4	4	5	4	4	3	3	3	3	4
	49	5	5	6	6	5	3	3	3	3	4
Spermine	0	22	22	22	22	22	24	24	24	24	24
	3	25	22	22	24	22	22	22	21	22	20
	7	16	10	27	24	27	17	19	24	22	22
	21	19	21	30	26	31	19	20	21	22	20
	35	28	29	29	28	29	21	24	24	26	24
	49	38	32	35	35	33	24	28	24	26	29

incubation, whereas GS11 showed only a slightly decreased growth curve as compared with the 1% GDL addition. Histamine was not produced by the strains GS-11 and G-261 in any of the broth compositions studied. G-106 produced 1 ppm histamine in pure MRS broth, 2 ppm in pure MRS broth at pH 4.7 and 11 ppm in MRS + GDL. Addition of histidine resulted in a great increase of histamine in MRS+H (5964 ppm) and MRS+H (pH 4.7) (6120 ppm). However, in MRS+H broth with GDL addition only 55 ppm of histamine was detected.

Table 2. pH values in sausages during fermentation in two trials. The starter cultures were Baktoferment 61 with 0.7% glucono-delta-lactone (GDL) and 0.15% glucose (B), Duploferment 66 (1), Pentoferment 85 (2), Condi-Rasant 820/10 (3) and Flora-Carn SL (4) with glucose/GDL combinations of 0.6% glucose (a), 0.4% glucose + 0.25% GDL (b) or 0.2% glucose + 0.5% GDL. * = not studied.

Code	Day 0	Day 1	Day 3	Day 7	Day 21	Day 35	Day 49
First trial:							
B	5.6	4.6	*	4.6	5.0	4.9	4.8
1a	5.6	5.8	*	4.8	5.0	5.0	4.9
2a	5.6	5.7	*	4.8	5.0	4.9	4.9
3a	5.6	5.7	*	4.4	4.8	4.8	4.6
4a	5.6	5.8	*	4.8	4.9	4.9	4.9
Second trial:							
B	5.5	5.0	5.1	4.7	4.5	4.4	4.7
1a	5.5	5.5	5.4	4.8	4.6	4.5	5.1
2a	5.5	5.5	5.5	4.9	4.6	4.4	4.9
3a	5.5	5.6	5.1	4.7	4.6	4.3	4.8
4a	5.5	5.6	5.3	4.8	4.6	4.5	4.8
1b	5.5	5.4	5.3	4.8	4.6	4.5	4.8
2b	5.5	5.4	5.4	4.8	4.6	4.2	4.8
3b	5.5	5.3	5.1	4.7	4.6	4.3	4.8
4b	5.5	5.4	5.2	4.8	4.7	4.6	4.8
1c	5.5	5.2	5.2	4.9	4.7	4.5	4.8
2c	5.5	5.2	5.2	4.8	4.6	4.5	4.8
3c	5.5	5.2	5.1	4.8	4.6	4.5	4.8
4c	5.5	5.2	5.1	4.8	4.7	4.5	4.9

Table 3. Results of microbiological studies during the ripening of dry sausages (log_{10} cfu/g) in two trials. B and the numbers 1-4 refer to the different starter cultures used, see Table 2. * = not studied.

		First trial:					Second trial:				
	Day	B	1a	2a	3a	4a	B	1a	2a	3a	4a
Lactic acid bacteria	0	3.8	3.8	3.8	3.8	3.8	5.0	5.0	5.0	5.0	5.0
	3	*	*	*	*	*	*	*	*	*	*
	7	7.8	7.8	7.8	8.5	7.9	*	*	*	*	*
	21	7.7	7.5	7.8	8	7.5	8.3	8.6	8.7	8.2	7.7
	35	7.7	7.5	7.6	7.7	7.4	*	*	*	*	*
	49	7.5	6.9	7.1	7.2	7	8.5	8.6	8.1	7.8	7.7
Staphylococcus sp.	0	*	*	*	*	*	*	*	*	*	*
	3	*	*	*	*	*	*	*	*	*	*
	7	5.7	5.6	6	6.9	6.9	*	*	*	*	*
	21	5.7	5.6	5.7	6.7	6.7	5.2	5.8	5.5	6.3	6.3
	35	5.5	5.6	5.4	6.1	6.1	*	*	*	*	*
	49	5.7	5.3	5.8	6.5	6.5	5.1	5.5	5.3	5.5	5.7

Table 4. pH values of two non-starter lactic acid bacteria strains (G-106 and G-261) and one starter lactic acid bacteria (GS-11) during incubation in six different combinations of de Man-Rogosa-Sharpe broth (MRS), histidine (H) (2%) and glucono-delta-lactone (GDL) (1%).

	MRS pH 6.2	MRS + H pH 5.4	MRS pH 4.7	MRS + H pH 4.7	MRS + GDL	MRS + H + GDL
G-106						
1 h	6.2	5.4	4.7	4.7	4.9	4.6
5 h	6.2	5.4	4.7	4.6	4.7	4.5
21 h	4.6	4.5	4.6	4.5	4.5	4.5
27 h	4.3	4.4	4.6	4.3	4.5	4.4
120 h	3.7	4.1	4.4	4.3	4.1	4.0
G-261						
1 h	6.2	5.3	4.6	4.7	4.8	4.6
5 h	6.2	5.3	4.6	4.7	4.7	4.5
21 h	4.4	4.3	4.6	4.5	4.5	4.4
27 h	4.2	4.1	4.5	4.3	4.5	4.4
120 h	3.8	3.6	4.3	3.8	4.3	4.0
GS-11						
1 h	6.2	5.3	4.6	4.7	4.8	4.5
5 h	5.9	5.3	4.6	4.7	4.7	4.5
21 h	3.9	3.8	4.1	3.9	3.8	3.7
27 h	3.8	3.7	3.9	3.8	3.7	3.6
120 h	3.7	3.6	3.6	3.5	3.5	3.5

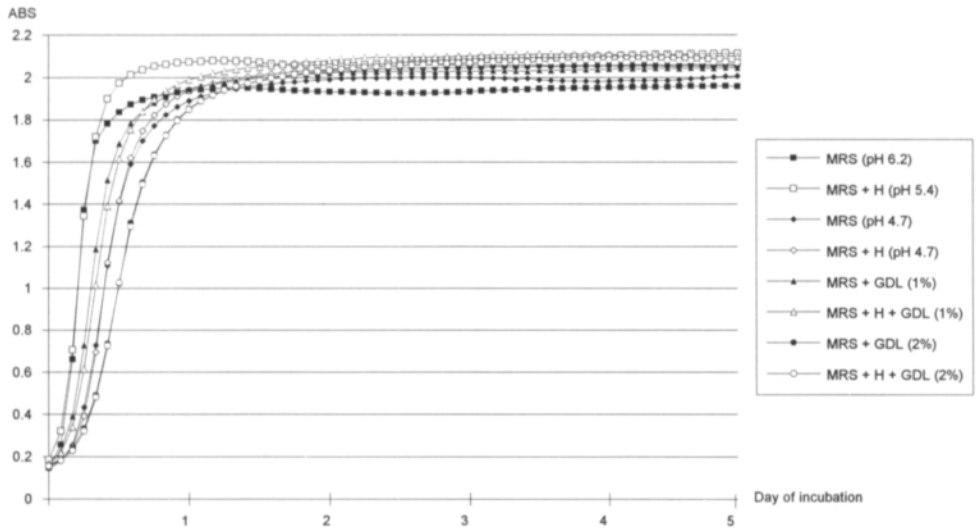

Fig. 3. Growth of strain GS-11 in eight different broth compositions.

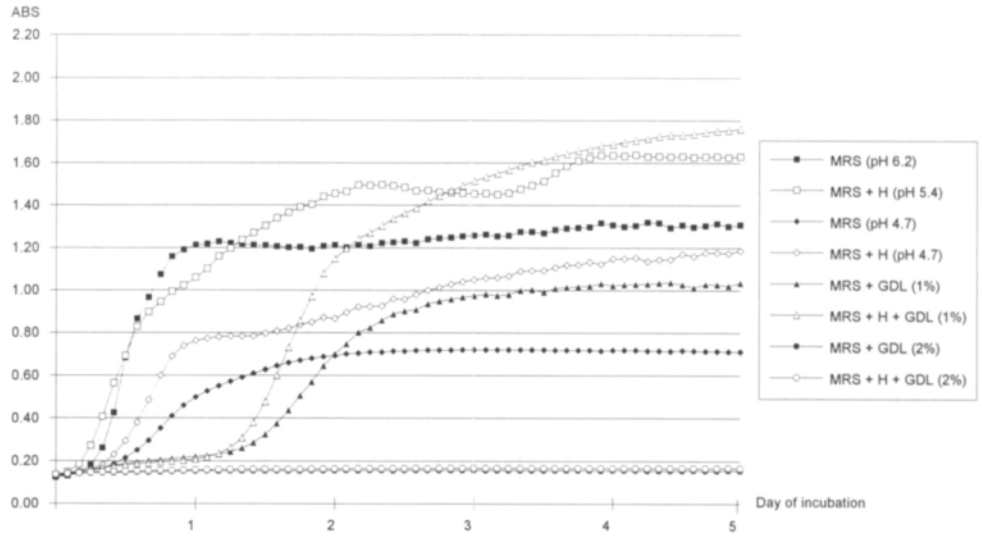

Fig. 4. Growth of strain G-106 in eight different broth compositions.

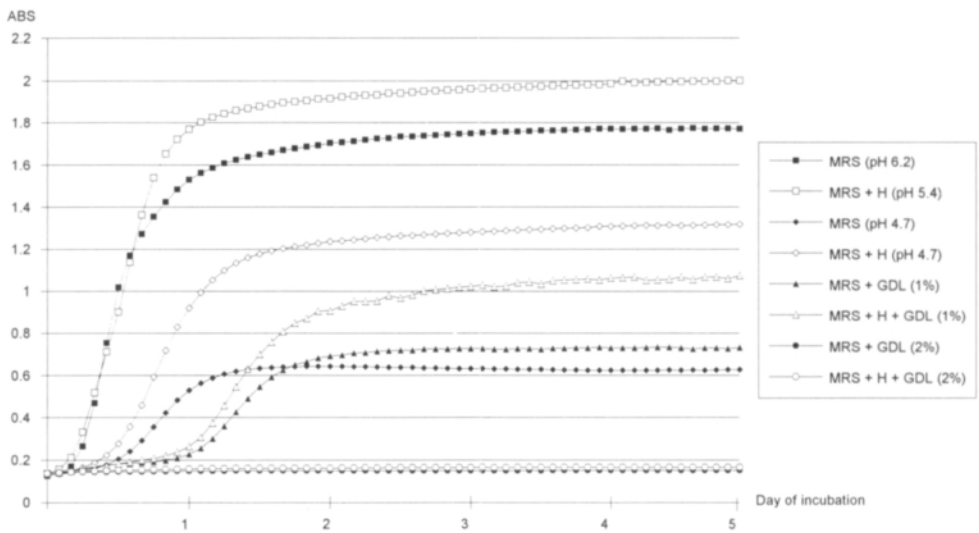

Fig. 5. Growth of strain G-261 in eight different broth compositions.

Discussion

In this study, histamine was only detected in the second trial, in which the number of LAB was higher than in the first trial. This conforms with the results of KRANNER et al. (1991). These authors reported that the formation of undesirably high amounts of histamine is dependent on a sufficiently high number of histamine-producing microorganisms and on the presence of free histidine as substrate. Furthermore it is influenced by the storage temperature and by the freshness of the meat used for production. TSCHABRUN et al. (1990) also found more LAB and total bacteria in dry sausages with

high histamine content than in those with low histamine. These authors could reduce the histamine content also by using very fresh meat.

EITENMILLER et al. (1978) reported that the use of *Pediococcus cerevisiae* decreased the formation of tyramine in dry sausages. On the basis of brand-specific studies of retail sausages, TAYLOR et al. (1978) also proposed that proper control of natural fermentation could largely prevent histamine formation. In the present study there were no great differences between the starter cultures studied concerning amines formed during ripening. However, it was interesting that even if the starter cultures used were histamine- and tyramine-negative *in vitro*, the lowest levels of histamine were found in sausages fermented by Baktoferment 61 + GDL or by the starter cultures containing LAB with GDL addition.

This phenomenon may be explained by the fact that the amine-positive NSLAB strains did not tolerate acidic conditions as well as the aminenegative starter culture in pure culture studies. This is reasonable because one of the most important criteria for the selection of starter strains has been their good lactic acid production capacity coupled with acid tolerance. Because the accelerated pH decrease caused by GDL addition increased the lag time and decreased the growth rate of NSLAB strains, it is probable that their number will remain lower for a longer time in sausages with GDL addition. This gives the starter culture the opportunity to outgrow the histamine-positive NSLAB strains. Because the main increase in histamine is detected during the first two weeks (TSCHABRUN et al. 1990) it is probable that the decrease in the number of e.g. histamine-positive strains during this period decreases the histamine levels detected in the final product.

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SELOSTUS

Eräiden herätеviljelmien ja glukono-delta-laktonin vaikutus biogeenisten amiinien muodostumiseen kestomakkaroissa

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Eläinlääkintä- ja elintarvikelaitos ja Eläinlääketieteellinen korkeakoulu

Tutkimuksessa selvitettiin viiden herätеviljelmän ja glukonodelta-laktonin (GDL) vaikutusta biogeenisten amiinien muodostumiseen kestomakkaroissa fermentaation aikana. Makkarat valmistettiin pilottehtaassa kahdesta eri raaka-aine-erästä ja niitä fermentoitiin 7 vk ajan. Herätеviljelmien välillä ei todettu suuria eroja biogeenisten amiinien pitoisuuksissa. Matalimmat histamiinimäärät olivat makkaroissa, jotka oli fermentoitu GDL:n ja stafylokokkien avulla yhdessä tai ilman maitohappobakteereita. Raaka-aineista peräisin olevien maitohappobakteerien todettiin puhdasviljelmätutkimuksissa olevan herätеviljelmäkantaa herkempiä happamille olosuhteille de Man-RogosaSharpe -liemessä (MRS-liemi). Histidiinilisä (2 %) sai aikaan selvän histamiinin tuoton kasvun histamiinipositiivisella kannalla (1-2:sta mg/l 6000:aan mg/l). GDL:n lisääminen histidiinipitoiseen liemeen vähensi kuitenkin histamiinin muodostumista selvästi (54 mg/l). Näiden tulosten perusteella näyttää siltä, että nopeutettu pH:n lasku GDL:n avulla vähentää histamiinin muodostumista kestomakkaroissa ja MRS-liemessä. Erot pH:n laskussa voivat olla yksi selittävä tekijä kauppamakkaroissa todettujen histamiinipitoisuuksien suurelle vaihtelulle.